Male sterility in *Corymbia calophylla* (marri)

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Most *Corymbia calophylla* trees have inflorescences of hermaphrodite flowers with a variable number of male flowers. Some trees have been identified that have either only female flowers, or a seasonally variable production of female flowers at the beginning of the flowering season, changing to production of hermaphrodite flowers after the first 2–3 weeks of flowering. In the flowers of the female trees, the anthers develop normally until the stage of meiosis when premature disintegration of the tapetum is followed by degeneration of the pollen mother cells. Female trees appear to be rare but widespread.

KEYWORDS: Female flower; Pollen sterility.

INTRODUCTION

Early illustrations of buds and flowers of marri (*Eucalyptus calophylla* (Lindl.) KD Hill & LAS Johnson) such as those by Baker and Smith (1920, Fig. 2 in Carr et al. 1971) showed flower buds of two shapes – some pear- shaped and others more spherical or top-shaped. This remained unremarked by botanists until Carr et al. (1971) reported that the pear-shaped buds were hermaphrodite, while the spherical ones were male, i.e. female sterile. Earlier observers may have thought the degenerated ovary contents in spherical buds were a result of fungal or insect damage, but this is not the case with most corymbs having some male flowers amongst the hermaphrodite ones. The proportion of male flowers varies and some trees have only male flowers (Carr *et al.* 1971). The variety *maideniana* Hochr. from the Darling Range, was shown to be based on a type specimen with only male flowers (Carr & Carr 1972).

A further variation of floral structure is reported here. During collection of pollen for hand pollinations, some marri trees were identified that had only female flowers.

METHODS

An attempt to collect pollen from newly opened buds of marri in the Kalamunda hills and foothills resulted in the identification of a number of trees that produced no pollen. Anther development was examined to determine the cause of the male sterility. Hermaphrodite and male sterile buds at different stages of development were collected (Table 1), fixed in formalin acetic alcohol, processed for sectioning in paraffin wax, cut at 8 μm and stained in 0.5% toluidine blue . Buds were fixed over the period 14th December 2013 to 4th April 2014.

Observations were made over several years to determine the consistency of expression of male sterility.

Herbarium specimens were examined at the Perth herbarium and for those without pollen obvious on the anthers, anthers of flowers or unopened buds were crushed in aceto-orcein and examined for the presence of pollen.

RESULTS

In a number of Kalamunda trees initially only female flowers (i.e. male sterile flowers), were present but after one to two weeks the trees produced hermaphrodite ones. The initial occurrence of female flowers varied with season. Four trees were identified that produced only female flowers over the entire flowering season, at least to the height of 5 m. This was consistently the case for two of the trees that were observed over the flowering periods of 2013 to 2016 which included years of abundant flowering as well as sparse years. A third female tree did not flower in 2015, and the fourth tree was burned in 2015 and did not flower in 2016. The female trees set the usual crop of capsules and seeds. Trees with female flowers also had some female sterile flowers in their corymbs making these flowers totally sterile.

The female flowers on living trees and herbarium specimens were generally smaller than the hermaphrodite ones (Figure 1) and this size difference is the best indication that a tree may be female. Anthers of female flowers after anthesis, in living specimens and herbarium specimens are pale and small, but otherwise similar to those of male or hermaphrodite flowers from which the pollen had been shed and collected by insects. Thus to identify a female flower it is necessary to examine the anthers of mature unopened buds. It is possible that the herbarium collections referred to as 'female' could be from trees that later in the season produce hermaphrodite flowers, but the flower size would suggest they are from female trees.

The anthers from hermaphrodite buds had darkly stained premeiotic pollen mother cells with a well defined tapetum. During meiosis (Figure 2A) and through to the stage of tetrads (Figure 2B) and uninucleate pollen, the tapetum was still in place although becoming vacuolated. The mature anthers showed the normal development of the fibrous layer and

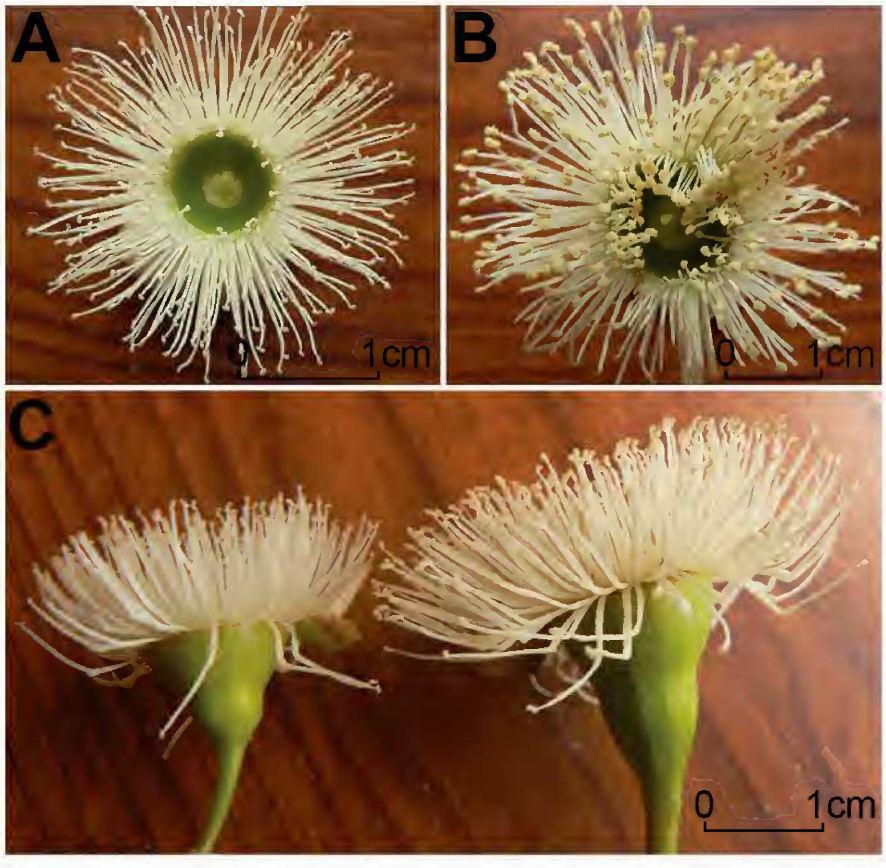

Figure 1. *Corymbia calophylla* trees from Kalamunda. A. Female flower, B. Hermaphrodite flower. C. Left female, Right Hermaphrodite flowers.

Table 1. *Corymbia calophylla* trees: locations and flower fertility

Location/herbarium number	Sex of flowers	Histology
Remnant tree on road verge at No 20 James Rd. Kalamunda.	Hermaphrodite and male, 2013–16	Sections of hermaphrodite flowers
Remnant tree on road verge of 29 Betti Rd (between James and Grace corners) Kalamunda	Hermaphrodite and male 2013–2016. Initially female in first weeks of flowering, 2013, 2016.	Sections of hermaphrodite flowers
Remnant tree on road verge SW corner of Robbins Rd and Grace Rd. Kalamunda.	Female , 2013–14, 16	Sections of female flowers
Remnant tree on road verge at No 5 Betti Rd. Kalamunda. (PERTH 08733619)	Female 2013–16	Sections of female flowers
Remnant tree on road verge at No 18 James Rd Kalamunda (PERTH 08733627)	Female 2015, 16.	
Tree in a remnant patch of trees at corner of Crystal Brook Road and Welshpool Road, Wattle Grove	Female 2015.	
Herbarium specimen Between Dunsborough and Cape Naturaliste (PERTH 01319256)	Female 1982	
Herbarium specimen Marangup Reserve off Toodyay Rd (PERTH 08029164)	Female 2007	

tricolpate pollen with dark stained deposits at each pore (Figure 2C).

In the anthers of the two female, (male sterile) trees sectioned, there were no major differences from male fertile anthers until the onset of meiosis. At meiosis in the female trees, the pollen mother cells rounded up and the tapetal cells were very faintly stained and more vacuolated than in the hermaphrodite trees. The most advanced meiotic stages were pachytene or diplotene (Figure 2D). Degeneration of the sporogenous tissues followed quickly (Figure 2E) with pollen mother cells becoming vacuolated then cell contents of each locule degenerating into a small deeply stained area. This reduced the size of the anther and distorted its shape, but the subepidermal fibrous layer developed normally with the empty locules splitting open at anther maturity

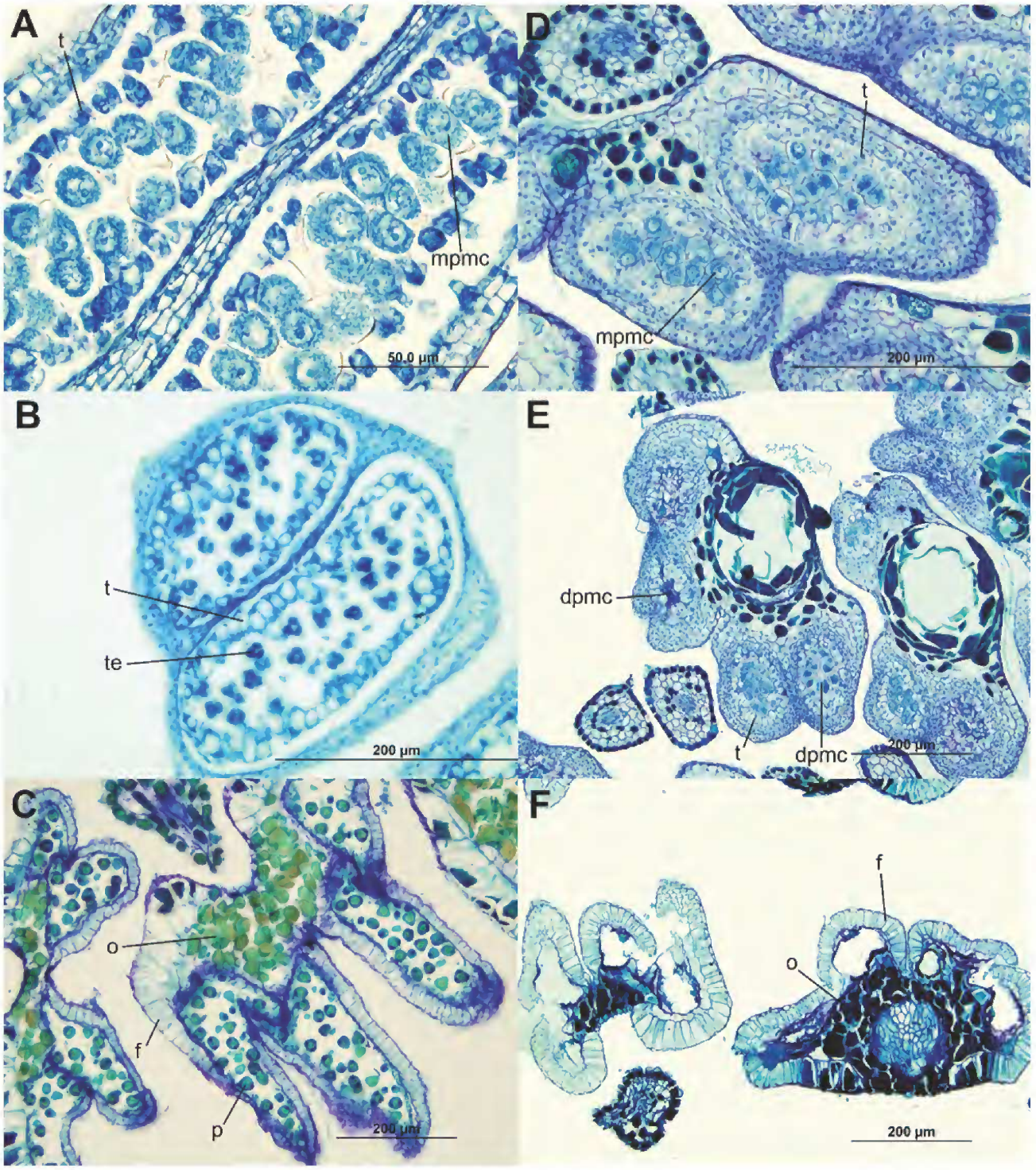

Figure 2. *Corymbia calophylla* anther development. Trees from Kalamunda A–C. Fertile anthers from hermaphrodite flowers. A, anthers at early prophase of meiosis, B, tetrad stage, C. Pollen in mature anthers. D–F. Sterile anthers from female flowers. D. anthers at early prophase, note vacuolate tapetum, E. degeneration of pollen mother cells. F. anthers with empty locules but mature walls with fibrous layer developed. t, tapetum, mpmc meiotic pollen mother cells, te tetrads, dpmc degenerating pollen mother cells, f fibrous layer , o oil cells, p pollen.

(Figure 2F). The development of the oil cells in the filament was similar to the fertile anthers.

The female trees set crops of capsules comparable to those on nearby hermaphrodite trees.

The two herbarium specimens observed to have only female flowers had short pale anthers and although there were a few small pollen grains on the surface of open flowers of the Dunsborough specimen, an unopened bud showed no pollen in the anthers and this tree is considered a putative female (Figure 3). Similarly no pollen was present in anthers from unopened flowers of the Toodyay specimen.

DISCUSSION

Flowers of marri are usually hermaphrodite or male, but female flowers and completely sterile flowers also occur. The production of female flowers may be transient at the beginning of the flowering season or a tree may produce only female flowers. In the female flowers the pollen mother cells and tapetum degenerate at the onset of meiosis, but the anther wall matures as in the fertile anthers. Tapetal abnormalities are frequently related to male sterility though mostly reported in herbaceous plants (Kaul 2012).

If the first flowers that open on a tree are female they must be outcrossed to set fruit, while in female trees all flowers will be outcrossed. Hermaphrodite flowers may be geitonogamously pollinated or outcrossed. Eucalypts, as with other forest trees, show inbreeding depression associated with self pollination (Sedgley and Griffin 1989).

The mix of male flowers, or sterile flowers in the corymb probably adds to its attraction for insects without being a sink for resources following seed set. The stigma of the hermaphrodite flowers does not become receptive until 7–9 days after anthesis (J. McComb data not shown) and this long period means that each corymb has a long lasting display of flowers.

Pryor (1976) reported that some trees of *E. pulverulenta* Sims had pollen with no protoplasmic content, and a detailed study by Peters *et al.* (1990) showed a wide range of pollen sterility in both a natural and cultivated population, with number of trees in the cultivated stand having no fertile pollen. Pryor (1976) also reported an unusual type of male sterility from *E. grandis*. In this case the pollen grain wall released no protein on to a stigma or a sucrose or agar gel and the grains failed to germinate.

While male flowers have previously been reported from *C. calophylla* and closely related species, as well as from other genera of Myrtaceae (Bentham, 1869), there are few reports of female flowers. *Melaleuca cornucopia* Byrnes is recorded as having female flowers and can be monoecious or gynoecious (Byrnes 1985).

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